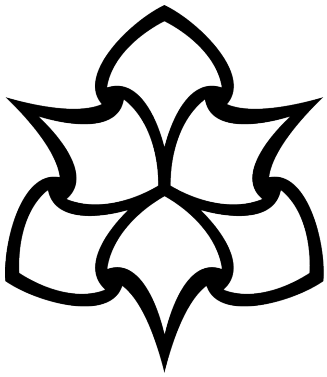

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Molecularly imprinted polymer based electrochemical biosensors: Overcoming the challenges of detecting vital biomarkers and speeding up diagnosis

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ABSTRACT

Electrochemical biosensors for the detection of vital biomarkers is a well-established technology that utilises a transducer and recognition element in tandem to determine the presence of an analyte. There is growing interest in using Molecularly Imprinted Polymers (MIPs) as recognition elements in a wide range of sensing devices due to their economic viability and scalability. The inherent properties of polymer platforms, alongside the vast array of monomeric options, synthetic routes and incorporation strategies allow for the production of a multitude of sensitive and selective recognition elements that have significant advantages over classically utilised biological entities. MIPs exhibit superior chemical and thermal stability offering a wider variety of immobilization/incorporation strategies, virtually unlimited ambient shelf-life and a longer product lifetime, whilst the vast array of monomers available offer flexibility to their synthesis. Even though some sensor platforms have been reported for the detection of vital biomarkers, the use of MIPs has a number of challenges and drawbacks that need to be overcome in order to produce sensing platforms with the required sensitivity and specificity for clinical use. In this review, we will provide an overview of the reasoning behind using MIPs as recognition elements in electrochemical biosensors for vital biomarkers, discuss the problems synergizing MIPs and electrochemical read-out strategies and offer insights into the future perspectives of this promising and innovative technology.

1. Scope of review

The development of electrochemical biosensors is an extremely popular, ever growing and diverse area of research, with over 2000 research papers published in 2019 alone, representing a growth of over 200% compared to where the field was in 2003 (see Fig. 1) [1]. Electrochemical biosensing for clinical biomarker analysis in-particular has received significant attention due to the rapid, low-cost and portable nature of electrochemical sensing. The rapid detection of clinical biomarkers for diagnosis and disease monitoring is particularly important in many clinical fields such as cancer, sepsis and cardiovascular disease (CVD) [2, 3], with a wide body of evidence demonstrating improved patient outcomes following early diagnosis [4–12]. Current testing approaches for the biomarkers related to these diseases traditionally utilise interactions between antibodies and antigens, with turnaround times ranging from hours to days, which can, in some cases, depending on the test, delay treatment and, potentially, negatively impact outcomes. Molecularly Imprinted Polymers (MIPs) are recognition elements that can be tailor-made synthetically to match a biomarker target. Research into the use of MIPs for biosensors is rapidly expanding (Fig. 1A), due to their superior chemical and thermal stability, versatility and low-cost of production compared to their conventional biological counterparts [13]. However, imprinting for the detection of proteins has been one of the most difficult areas in development and there are significant challenges that must be overcome to match the specificity and sensitivity of current technology and achieve their widespread use as a clinical diagnostics tool [14]. In this review, we highlight the work published in the area of developing electrochemical MIP biosensors for clinical biomarkers, critically analyse the challenges that are faced and present our opinion on potential future developments.

1.1. Importance of current methods for clinical biomarker detection

Cardiovascular disease (CVD), cancer and sepsis are all classified as leading causes of death globally, with CVD ranking as the number one

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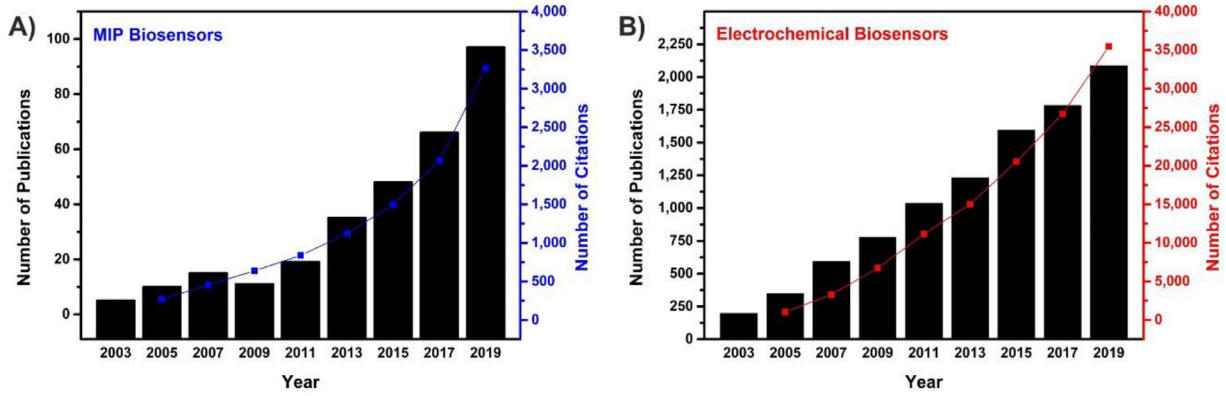

Fig. 1. A) Number of publications and number of citations per year for Molecularly Imprinted Polymer Biosensors based on results search and citation report for "Molecularly Imprinted Polymers" and "Biosensor" on Web of Science [1] conducted on 04/06/2020. B) Plot of the number of publications and number of citations per year for Electrochemical Biosensors based on results search and citation report for "Electrochemical" and "Biosensor" on Web of Science [1] conducted on 04/06/2020.

cause [3]. CVD is responsible for an estimated 17.9 million deaths annually worldwide, with four out of every five CVD related deaths being a direct result of heart attacks (acute myocardial infarction) and strokes [15]. In the UK, it affects around 7 million people every year and has been reported to cost the NHS approximately £7 billion (GBP) to treat annually [16]. Collectively, cancers are the second leading cause of death globally, responsible for an estimated 9.6 million deaths (1 in 6) globally in 2018 [17]. There is a vast array of different cancers, requiring different biomarkers for some aspects of their diagnosis and monitoring. For colorectal and breast cancer, the cost to the NHS of treatment is significantly reduced with earlier diagnosis (stages 1 and 2), highlighting the demand for rapid point-of-care testing (POCT) to aid with prompt diagnosis and treatment [18]. The United Nations World Health Assembly has recognised sepsis as a global health priority. More than 19 million sepsis cases and 6 million sepsis-related deaths are estimated to occur annually. In the UK, the annual number of admissions (77,996) and recorded deaths (15,851) related to sepsis has dramatically risen in recent years; a 41% and 38% rise between 2015 and 2017 respectively [2]. The condition is reported to cost the NHS in England approximately £2 billion annually [19]. It has been reported that over 34% of Intensive Care Unit (ICU) patients develop sepsis at some point during their stay and 27% of patients with sepsis die, rising to over 50% in patients with septic shock [20]. Again, the importance of rapid diagnosis is clearly demonstrated by evidence highlighting high mortality rates in the absence of prompt treatment; indeed, it has been reported that for every hour that sepsis is not identified and treatment is not initiated, there is a 7.6% reduction in survival rate [21]. Sepsis and cardiac dysfunction can be directly linked through a condition known as Sepsis-Induced Cardiomyopathy (S-IC) in which the widespread inflammation occurring during sepsis negatively affects cardiovascular performance. Approximately 50% of patients with sepsis develop S-IC [22], and this is associated with a significantly increased mortality to that of sepsis without this complication. Therefore, strategies to improve the early identification of sepsis and its complications may reduce the severity and economic burden of the condition. Indeed, it is estimated that 11,000 lives and £160 million could be saved every year through improved diagnosis and treatment [19].

Prior to the outbreak of SARS-CoV-2 (COVID-19), the POCT market was expected to grow in the US to $36.96 billion in 2021 corresponding to a compound annual growth rate (CAGR) of 9.8% [23]. This aligns well with the reported projected CAGR for electrochemical biosensors of 9.7% between 2016 and 2022; with a market value of approximately $33 billion by 2027 [24]. Electrochemical immunoassays are the most commonly reported form of electrochemical biosensors for detecting clinical biomarkers. Classically, this involves the use of specific antibodies as a recognition element in conjunction with an electrode acting as a transducer. The binding phenomena between an antibody and antigen can then be readily detected through various electrochemical techniques. Numerous reports of these biosensors have been made for MI, cancer and sepsis utilising a variety of electrode materials and designs [25–32]. However, many of these suffer in terms of sensitivity, selectivity or reproducibility due to common drawbacks with this methodology such as the quality of the purchased antibodies, the reliability of the immobilisation methodologies, the orientation of the antibodies on the transducer, difficulties producing results in biological matrices and the lifetime and storage of produced devices [33, 34]. Many of these challenges come directly from the use of antibodies; as such, there has been a focus on replacing these with synthetic recognition elements called Molecularly Imprinted Polymers (MIPs). These polymers offer many possible advantages over the use of biological recognition elements as they offer superior chemical and temperature stability, the ability to tailor the synthesis to the target molecule and allow for cheaper production [35, 36].

2. Molecularly imprinted polymers

The first English report of Molecularly Imprinted Polymers (MIPs) was produced in 1949 by Dickey, who introduced some of the key concepts used in MIP development today, such as identification of the target molecule as a "template" [37]. The more commonly seen non-covalent imprinting that is predominantly used in current research was first introduced by the group of Mosbach in the 1980's [38–40]. The general description of MIPs is that of a polymeric matrix with distributed binding cavities that are specific for the size, shape and functionalities of the imprinted template. They are generally formed through a generic sequence of events, as shown schematically within Fig. 2:*1)* the incubation of monomers with either a template molecule, dummy template or an epitope, allowing for non-covalent interactions to form between the functional monomers and the template and for them to stabilise; *2)* the polymerisation of the monomers to form a polymer around the template, effectively trapping it in the polymeric matrix; *3)* the removal or extraction of the template from the polymer to leave specific binding cavities of the same size and shape for that template.

The interactions between monomers and templates can be studied prior to synthesis through computational modelling, which can dictate or be dictated by synthesis choice [41]. How the polymerisation procedure is accomplished can vary greatly between the vast array of synthetic routes for MIP formation, such as UV polymerisation [42], thermal polymerisation [43], Reversible Addition–Fragmentation chain Transfer (RAFT) polymerisation [44] and solid-phase synthesis [45] to

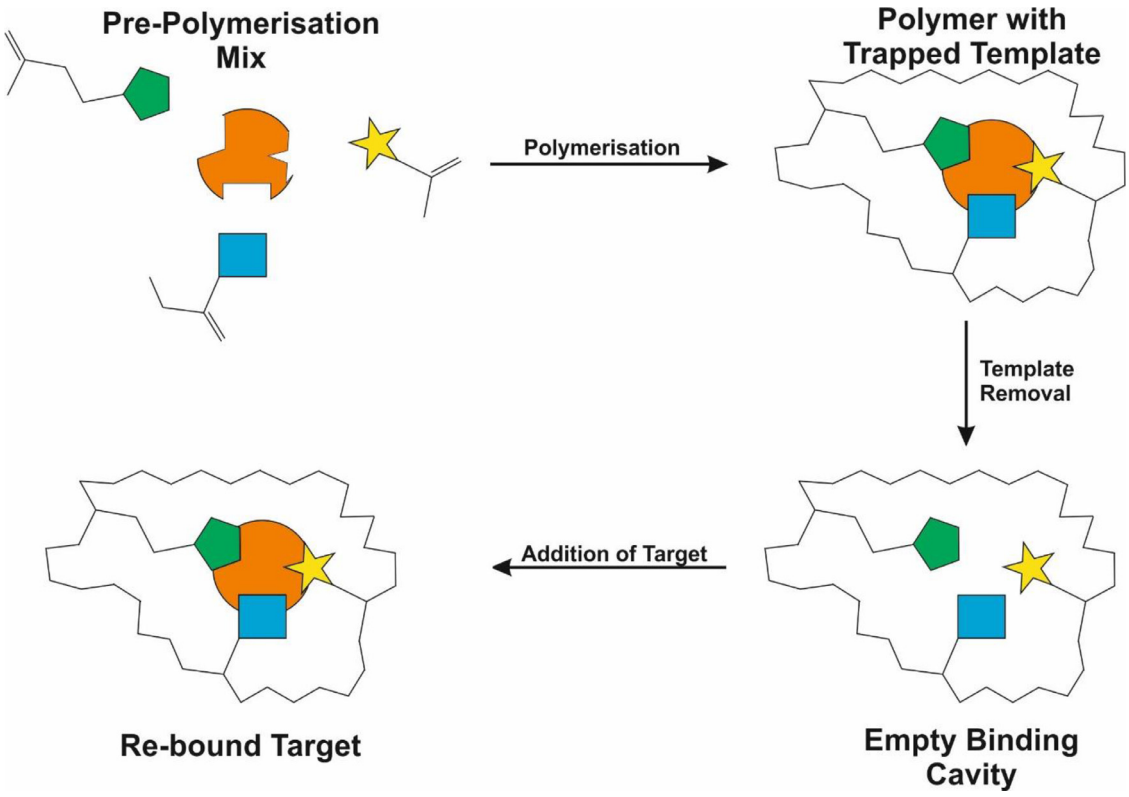

Fig. 2. Schematic of the general production methodology for a Molecularly Imprinted Polymer. A pre-polymerisation mixture allows for the non-covalent interactions to stabilise between the template and the functional monomers. The polymerisation process then freezes the template inside the polymer matrix. The template is then removed from the polymer leaving a binding cavity of complementary size, shape and functionality to the target. When the target is introduced it will bind back inside the cavity.

name a few; more detailed explanations of these processes can be found in the following reviews: [46–49]. However, traditional bulk imprinting has proved challenging for proteins due to their reduced mass transfer, permanent entrapment in the polymer matrix, poor structural integrity of the polymer, restricted synthetic routes especially in solvent selection and the heterogeneity of binding sites [50–52]. All the different routes for MIP synthesis can lead to significant differences in the properties and morphologies of the final product. This can be advantageous for tailoring production but has led to a vast majority of systems struggling to achieve the synergy, selectivity and sensitivity needed to perform at the required standards in sensor platforms.

The majority of reports utilising a combination of MIPs and electrochemical detection use electropolymerization as their chosen method of MIP formation [53, 54]. This synergises well with the overall platform due to the possibility of producing both conductive and non-conductive polymers (depending on the desired platform and electrochemical detection method) and the direct formation of the MIP layer on the surface of the transducer, effectively removing a time-consuming immobilisation step. This methodology is based on the formation of polymers through the application of potential, causing an oxidation or reduction in the monomers and has been applied to the detection of a range of biological targets [55]. Although convenient, the formation of MIPs through electropolymerization has significant challenges to overcome such as the more limited supply of suitable monomers, the homogeneity of the binding sites produced and the scalability of the process. Other methods of synthesis that produced superior performing MIPs typically struggle with reproducibility of immobilization techniques or synergising with the read-out technique; however, some excellent examples of MIP sensors have been achieved for biological targets using dip-coating [56, 57], screen-printing [43, 58] or a sol-gel approach [59–61]. In order to achieve the levels of detection and reliability required for a successful sensor for clinical biomarkers, careful optimisation of the synthesis, template removal and detection parameters must be carefully applied. Throughout this review, we highlight some of the recent reports of electrochemical MIP sensors for clinically relevant biomarkers and discuss how they achieved their goal or how they could be improved in future work.

3. Biomarker detection

3.1. Acute myocardial infarction (AMI)

AMI, commonly known as a heart attack, occurs when there is a decrease or stop in the flow of blood to a part of the heart that leads to myocyte necrosis [62]. In 1999, the Joint European Society of Cardiology/American College of Cardiology Committee released a new definition for AMI that emphasised the importance of biomarkers in the diagnosis, introducing cardiac troponins (cTn) as the gold standard [63]. This was updated in 2012 and 2018, both times emphasising the role of cTn levels in the diagnosis of AMI [64, 65]. Cardiac troponins T and I (cTnT and cTnI) are encoded by different genes, which makes them immunologically distinct [66]. Both have been reported as specific and sensitive biomarkers for AMI and superior to creatinine kinase-MB and myoglobin as indicators for myocardial necrosis. Some novel emerging biomarkers include heart-fatty acid binding protein (h-FABP), B-type natriuretic peptide (BNP), ischaemia-modified albumin (IMA), pregnancy-associated plasma protein A (PAPP-A), copeptin and growth differentiation factor-15 (GDF-15) [67]. In the UK, the National Institute for Health and Care Excellence (NICE) committee, recommends two troponin assays (Roche Elecsys® measures troponin-T levels and Abbott's Architect STAT Troponin-I assay) for the rule-out of AMI in patients with suspected heart attack. These tests measure troponin levels in serum at

Table 1
Summary of MIP based electrochemical sensors for cardiac biomarkers, highlighting the polymer used, electroanalytical method of detection, linear range and limit of detection.

Target	MIP	Electrode Material	Detection Method	Linear Range	Limit of Detection	Reference
cTnT[a]	*o*-PD[c]	Au[m]	CV[r]	0.009–0.8 ng mL^{-1}	9 pg mL^{-1}	[75]
cTnT[a]	PANI[d]	SPCE[n]	DPV[s]	0.02–0.09 ng mL^{-1}	0.008 ng mL^{-1}	[78]
cTnT[a]	PANI[d]	SPCE[n]	DPV[s]	0.1–8.0 pg mL^{-1}	0.04 pg mL^{-1}	[80]
cTnT[a]	AAM[e]	MWCNTs[o]	Potentiometric	–	0.16 µg mL^{-1}	[70]
cTnT[a]	PPy[f]	SPCE[n]	DPV[s]	0.01–0.1 ng mL^{-1}	0.006 ng mL^{-1}	[79]
cTnI[b]	PPy[f]	GCE[p]	DPV[s]	0.01– 5.0 ng mL^{-1}	0.0005 ng mL^{-1}	[83]
cTnI[b]	*o*-AP[g]	GCE[p]	DPV[s]	0.05–5 nM	0.027 nM	[82]
cTnI[b]	MB[h]	GCE[p]	DPV[s]	0.5–3.3 × 10^{5} pM	1.04 pM	[85]
cTnI[b]	MAA[i]	GCE[p]	DPV[s]	0.005–60 ng cm^{-3}	0.0008 ng cm^{-3}	[84]
Myoglobin	*o*-AP[g]	Au-SPE[q]	SWV[t]		0.8 µg mL^{-1}	[93]
Myoglobin	IL[j]	GCE[p]	CV[r]	0.06–6 µM	9.7 nM	[96]
Myoglobin	AAM[e]	Au-SPE[q]	EIS[u]	0.852–4.26 µg mL^{-1}	2.25 µg mL^{-1}	[92]
Myoglobin	MMA[i]	Au[m]	DPV[s]	1 x 10^{-10}–0.1 mg L^{-1}	1.1 x 10^{-11} mg L^{-1}	[97]
Myoglobin	Phenol	Au-SPE[q]	DPV[s]	0.001 ng mL^{-1}–100 µg mL^{-1}	2.3 pg mL^{-1}	[90]
Myoglobin	*o*-PD[c]	SPCE[n]	DPV[s]	1 nM–1 µM	0.5 nM	[98]
Myoglobin	SSA[k]/AEHM[l]	Au-SPE[q]	SWV[t]	–	0.79 µg mL^{-1}	[91]
Myoglobin	AAM[e]	Au-SPE[q]	EIS[u]	1–20,000 ng mL^{-1}	0.83 ng mL^{-1}	[89]

[a] cardiac troponin T, [b] cardiac troponin I, [c] o-phenylenediamine, [d] polyaniline, [e] acrylamide, [f] polypyrrole, [g] o-aminophenol, [h] methylene blue, [i] methacrylic acid, [j] ionic liquid, [k] 4-styrenesulfonic acid, [l] 2-aminoethyl methacrylate hydrochloride, [m] gold, [n] screen-printed carbon electrode, [o] multi-walled carbon nanotubes, [p] glassy carbon electrode, [q] gold screen-printed electrode, [r] cyclic voltammetry, [s] differential pulse voltammetry, [t] square wave voltammetry, [u] electrochemical impedance spectroscopy.

various time points and measured high levels, along with a typical rise and fall pattern of troponin, are strongly associated with cardiac events. However, there are significant limitations associated with these assays which include high cost and long measurement time due to the assay requiring a lab environment, which can have a negative impact on patient outcome since this is strongly linked to time for diagnosis.

A comparison between thirteen commercial troponin assays showed good correlation between the methods, while correlation between cTnI and cTnT levels varied substantially which can lead to issues with standardization of measurements [68].

To avoid the issues around turn-around time, other European countries have adopted new platforms such as the Siemans Atellica® Solution, which received FDA clearance in 2018. This system combines immunoassays and clinical chemistry analysers to reduce measurement times. Although measurement times have been improved, a common problem throughout clinical use is the slow turnaround time for results, stemming from sample procurement, transport to labs, and subsequent analysis, putting a significant burden on already overstretched clinical staff. In addition, the demand for troponin tests has rapidly increased, as it has been identified as a prognostic marker for many conditions, including COVID-19 progression [69]. This has ignited the interest in suitable POC platforms for detection of cardiac troponins and myoglobin, summarised in Table 1, which will overcome many of these current limitations.

The first reported MIP electrochemical biosensors in this area was published in 2011 by Moreira et al. [70] who produced the MIP receptors, specific for cTnT, onto the surface of multi-walled carbon nanotubes (MWCNT). This reportedly utilised the key characteristics of MWCNT such as their high strength, stability, lack of swelling and large active surface areas [71]. In conjunction with MIPs this method has the potential to produce stable potentiometric sensor platforms with good compatibility between the transducer, modification and recognition element [72]. However, the sensor platform did not reach the required relevant detection levels of cTnT (1–5 ng/L) [73]. This is likely due to the lack of homogeneity of the MIP binding sites, which is a common trait among MIP platforms produced using this synthesis methodology. The most commonly utilised electrochemical detection methodology found in the literature is the suppression of redox probes, such as ferri/ferrocyanide through binding phenomena. This is demonstrated by Kariman et al. [74, 75] who produced a sensor platform specific for cTnT through the electropolymerisation of *o*-phenylenediamine (o-PD). This is becoming a more popular choice of monomer for the formation of electropolymerised MIPs due to the excellent stability of the produced film and mild conditions required for formation [76, 77]. The sensor performed well in buffered solutions, producing a LOD of 9 pg mL^{-1} and working range of 0.009–0.8 ng mL^{-1}; however, it struggled with increased noise on the signal when measuring in more complex media. There have been attempts to improve the response of MIP systems towards cTnT utilising this redox probe by incorporating nanomaterials into the sensor design, such as reduced graphene oxide [78, 79]. This modification is achieved through drop-casting and produced reproducible results in diluted serum. However, the incorporation of this nanomaterial adds extra manufacturing steps into sensor production, whilst still requiring sample pre-treatment for measurement. More recently, Phonklam et al. [80] utilised electropolymerisation to produce polyaniline (PANI) MIPs on MWCNT with polymethylene blue (PMB) for cTnT, (Fig. 3A and B). In this way, the redox probe was immobilized onto the surface of the electrode instead of being free in solution, which increases the suitability for a Point-of-Care (POC) system. PANI was chosen as the imprinting polymer as it is possible to finely tune the polymer layer thickness through controlling the parameters of deposition and charge passed [53].

Although PANI offers some excellent properties such as its electrical conductivity, stability, ease of deposition and low-cost; the structure of the monomer is simplistic and only offers a single protonated amine group that can form non-covalent interactions with the target molecule, such as the carboxylic acid functionality on a protein [81]. This will limit the amount of highly specific binding sites produced in the matrix and lead to questions about selectivity and reproducibility. However, in this work, the selectivity studies show promising results with little interference from proteins such as cTnI. The LOD obtained for cTnT in this work of 0.04 pg mL^{-1} is well below the required normal physiological range required, with the linear working range of the sensor reported as 0.1–8.0 pg mL^{-1}. This raises questions about real-world applications for the technology, as this will obviously require sample pre-treatment for it to be in the correct concentration range.

The amount of literature published on the detection of cTnI is more limited than that for cTnT. In general, the sensors developed in this area again rely on the use of the ferri/ferrocyanide redox probe described above, in conjunction with the MIP immobilised onto the electrode sur-

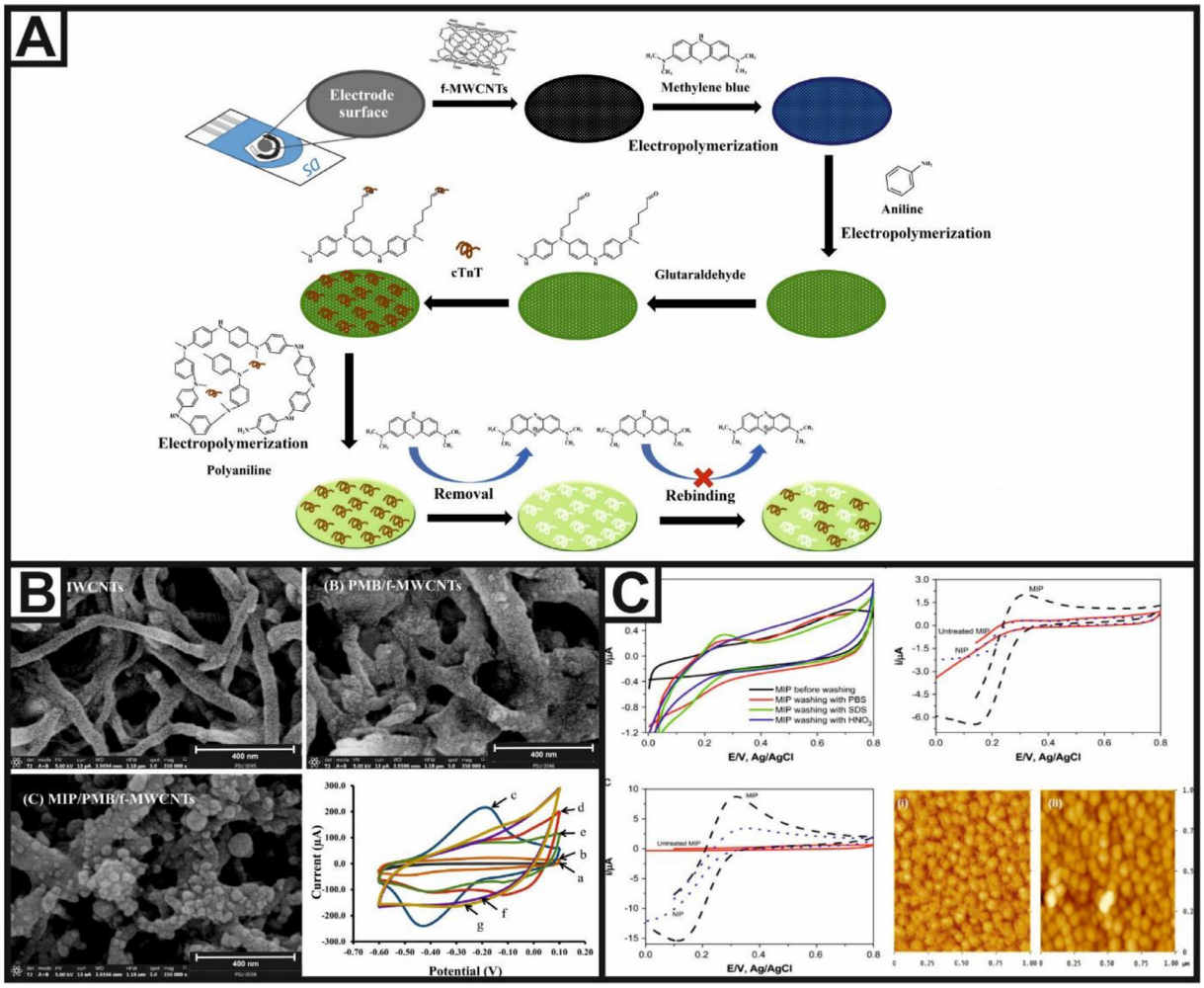

Fig. 3. A) Schematic illustration of the fabrication of a MIP based sensors for cTnT. Reproduced/adapted with permission from ref [80]. Copyright 2020 Elsevier. B) SEM images of MWCNT at different stages of sensor production with corresponding cyclic voltammograms. Reproduced/adapted with permission from ref [80]. Copyright 2020 Elsevier. C) DPV curves for the detection of cTnT with a MIP and NIP sensor combined with their corresponding sensing plots. Reproduced/adapted with permission from ref [74]. Copyright 2014 Elsevier.

face. There are examples of solutions relying solely on the MIP and an electrode [82], with the use of a single additive such as Boron Nitride Quantum Dots (BNQDs) [83] or with multiple added nanomaterials in a layer-by-layer fashion [84]. An interesting new methodology, that is receiving more recognition recently is the combination of MIPs with aptamers to form a hybrid sensing platform with dual recognition capabilities [85]. Aptamers are artificial nucleic acids or peptides that are designed for a specific target. When used in combination with MIPs they can help overcome the limitations that using solely non-covalent bonding presents [86–88]. In this way, the selectivity and specificity of aptamers are combined with the functionality, size and shape recognition of MIPs. Mokhtari et al. [85] bound aptamers specific for cTnI onto the surface of ZnO nanoparticles (ZnONPs), then immobilized these onto the surface of a GCE, Fig. 4A. Once the cTnI was bound to the aptamers, a layer of polymethylene blue (PMB) was electropolymerised around the aptamers/target complex. Following target removal, a hybrid recognition layer was left on the surface of the electrode combining a PMB MIP and aptamers. The binding of the target cTnI to the aptamers/MIP layer hinders the electron transfer from the PMB layer to the electrode surface producing a measurable decrease in the differential pulse voltammograms. In this way, the redox probe is encased in the sensing platform, and does not require an external solution change. This methodology offered a significant improvement in linear range over other reported systems from 0.5 to 3.3×10^5 pM, with a 1.04 pM LOD, which both meet the required LOD and biologically relevant analytical range for use. The issues with the system remain the transition from lab based sensor device to mass producible POCT, which is a common trait for MIP based electrochemical sensors utilising electropolymerisation as a key production method.

There is a more varied range of literature for the production and detection methods used for MIP based sensors targeting myoglobin. This is most likely due to the significant difference in purchase cost between the cardiac troponins and myoglobin, allowing for significantly more optimization of MIP composition at a lower cost per experiment. There are still several examples that utilise the ferri/ferrocyanide redox probe for their detection; relying on an inhibition in signal for either Differential-Pulse Voltammetry (DPV) [90], Square-wave Voltammetry (SWV) [91] or Electrochemical Impedance Spectroscopy (EIS) [92, 93]. EIS is a commonly explored detection strategy in conjunction with MIPs as it allows the user to monitor surface changes and complex binding events [94, 95]. The characteristic semi-circular plots, seen in Fig. 4B, represent the electron transport resistance or charge transfer resistance (R_{CT}). In the work presented by Karami et al. [89], the binding of myoglobin (or PSA) to the acrylamide based dual MIP sensor inhibits the transfer of charge through the interface to the electrode surface resulting in a measurable increase in the R_{CT}. This platform utilised photo-

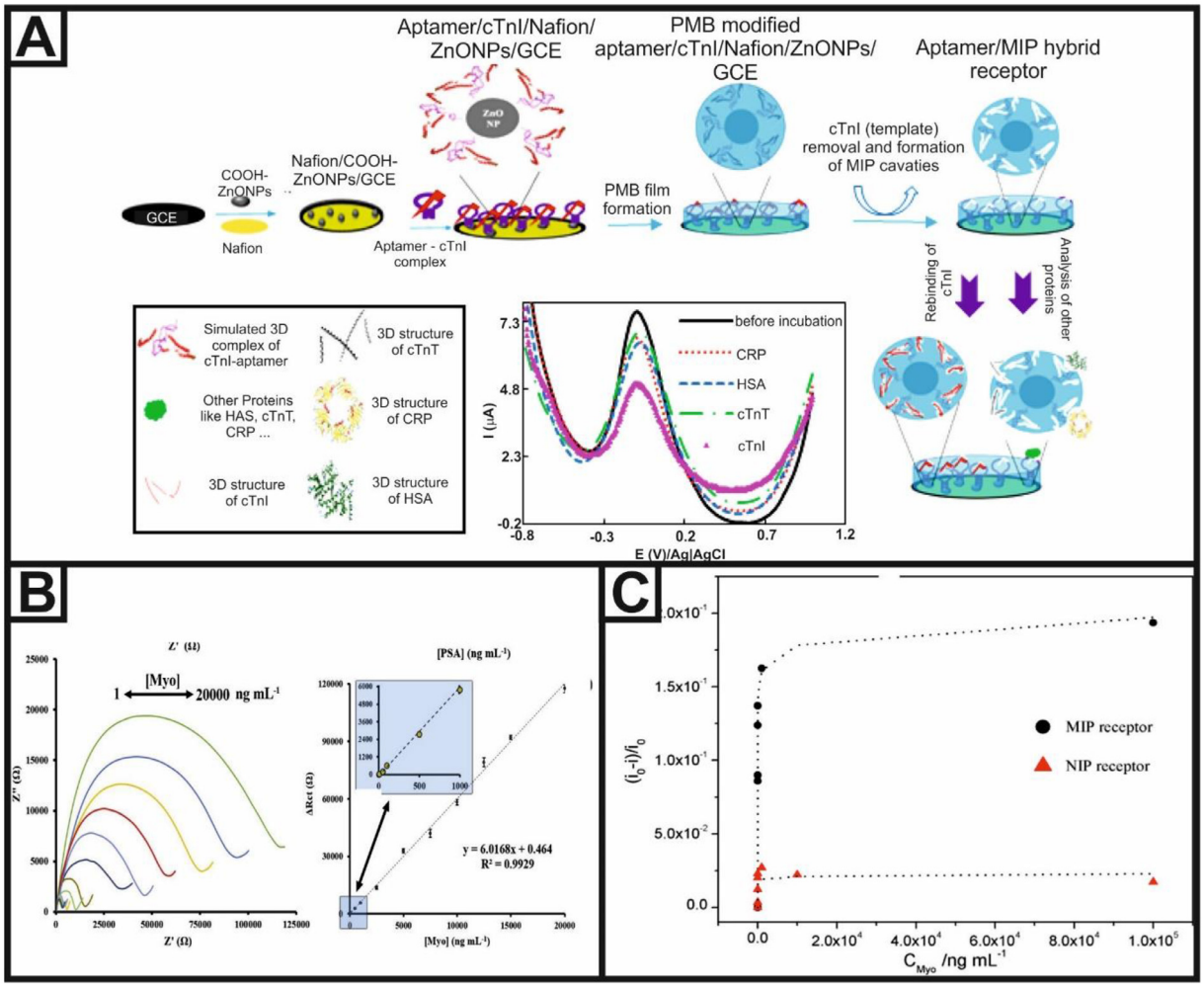

Fig. 4. A) Schematic representation of the aptamer/MIP receptor formation onto ZnONPs for cTnI detection. Reproduced/adapted with permission from ref [85]. Copyright 2020 Elsevier. B) EIS data for the MIP electrodes for incubation with myoglobin. Reproduced/adapted with permission from ref [89]. Copyright 2020 Elsevier. C) Comparison between the DPV response for the NIP and MIP for myoglobin. Reproduced/adapted with permission from ref [90]. Copyright 2017 Elsevier.

chemical polymerization with acrylamide as the functional monomer and N,N'-methylenebisacrylamide as the crosslinker monomer. Using this polymerization method produces a non-conductive polymer which will inherently hinder using electrochemical detection methods such as DPV or CV; whereas EIS offers a way around this. This sensor produced a low LOD of 5.4 pg mL^{-1} and wide working linear range of 1–20,000 pg mL^{-1}.

Although, the use of non-conductive MIPs with an electrochemical detection strategy seems counter intuitive, Ribiero et al. [90] have shown it is possible to produce a functioning sensor for the detection of myoglobin using DPV. They utilise the non-conductive polymer polyphenol, which as well as being known for electrode fouling, can be used to as recognition elements due to its ability to interact with molecules through π- π stacking. The issue with systems that use this method are the small linear ranges that can be achieved due to the small redox signal obtainable through a non-conductive polymer. Using EIS for a system like this could provide a wider working range to improve the system.

In terms of cardiac biomarker sensing using electrochemical MIP sensors, there is a plethora of examples using DPV in conjunction with a free redox probe; however, there are drawbacks when trying to convert laboratory work into real world POCTs. In addition, a balance needs to be struck between enhancing signals with nanomaterials and layer modifications and actual mass production capabilities. This includes the use of electropolymerisation and its suitability for mass producing POCTs.

3.2. Sepsis

Sepsis is a life-threatening condition caused by a dysregulated host response to infection. The infection is the instigator, while the host's immune system is responsible for the widespread organ damage characteristic of sepsis. If sepsis is not identified and treated rapidly, it will lead to multi-organ failure and death. While the definition of sepsis keeps on evolving [99], clinical assessment based on physiological and molecular biomarkers, plus the identification of blood stream infection (BSI) are crucial for early diagnosis and targeted management, yet these involve prolonged laboratory procedures (for the biomarkers) and the *in-vitro* detection or proliferation of bacteria, virus or other organisms which can take up to 72 h, or longer. Current UK guidelines for the early recognition and management of sepsis therefore include a combination of clinical judgement, observation of key physiological parameters via screening tools and consideration of patient risk factors and blood inflammatory markers (C-Reactive Protein (CRP), White Blood Cell Count (WBC), Procalcitonin (PCT) and others such as lactate and blood cultures (BC)). PCT and CRP are FDA approved for the assessment of progression of infection to sepsis, for aiding in decisions for antibiotic therapy for some patients, and also for the potential de-escalation of antibiotics for septic patients if tracked over time [100–102]. A heightened understanding of the inflammatory processes that lead to host tissue damage in sepsis has led to the identification of several other biomarkers with a high level of specificity to be identified. A hyper-inflammatory cytokine

Table 2
Summary of MIP based electrochemical sensors for sepsis and cancer biomarkers, highlighting the polymer used, electroanalytical method of detection, linear range and limit of detection.

Target	MIP	Electrode Material	Detection Method	Linear Range	Limit of Detection	Reference
CRP[a]	AEDP[g]/DMAA[h]	SPCE[n]	DPV[r]	–	0.04 μg mL^{-1}	[108]
IL-1β[b]	EBT[i]	SPCE[n]	EIS[s]	0.06– 600 nM	1.5 pM	[116]
IL-8[c]	3-APBA[j]	Au[o]	CV[t]	0.1–10 pM	0.04 pM	[115]
lactate	3-APBA[j]	SPCE[n]	EIS[s]	3–100 mM	1.5 mM	[109]
lactate	*o*-PD[k]	GCE[p]	DPV[r]	0.1 - nM	0.09 nM	[127]
lactate	3-APBA[j]	SPCE[n]	DPV[r]	10^{-6}–0.1 M	0.22 μM	[110]
CA-15[d]	*o*-AP[l]	Au-SPE[q]	DPV[r]	5–50 U mL^{-1}	1.5 U mL^{-1}	[123]
CA-15 [d]	TB[m]	Au[o]	DPV[r]	0.1–100 U mL^{-1}	0.1 U mL^{-1}	[122]
HER2-ECD[e]	Phenol	Au-SPE[q]	DPV[r]	10–70 ng mL^{-1}	1.6 ng mL^{-1}	[125]
PSA[f]	Dopamine	Au[o]	EIS[s]	0.1–100 ng mL^{-1}	1.0 pg mL^{-1}	[124]

[a] c reactive protein, [b] interleukin-1β, [c] interleukin-8, [d] cancer antigen 15, [e] human epidermal growth factor receptor – extracellular domain, [f] prostate specific antigen, [g] 2-acryl amidoethyldihydrogen phosphate, [h] N-(4-dimethylaminophenyl)-acrylamide, [i] eriochrome black T, [j] 3-aminophenylboronic acid, [k] o-phenylenediamine, [l] o-aminophenol, [m] toluidine blue, [n] screen-printed carbon electrode, [o] gold, [p]glassy carbon electrode, [q] gold screen-printed electrode, [r] differential pulse voltammetry, [s] electrochemical impedance spectroscopy, [t] cyclic voltammetry.

response [103], including production of IL-1β, IL-6, IL-8 and TNF-α, as well as products of cellular damage (*e.g.* High Mobility Group Box 1, HMGB1), has widespread effects on multiple organ systems. The ability to assess the patient's inflammatory phase in the clinical setting would therefore allow for improved risk stratification and treatment decision making. All of this makes the use of immunoassays toward the rapid diagnosis of sepsis interesting due to their ability to give results on the minute timescale, which would allow for the immediate start of treatment, potentially improving survival rates.

The development of biosensors for sepsis has been sparse in comparison to the more widely known diseases such as cancer and myocardial infarction, and there has been significantly less development in terms of MIP based electrochemical sensors, a compilation is presented in Table 2. All sensor platforms developed in the previous section for cardiac biomarkers are applicable to the detection of sepsis-induced cardiomyopathy (S-IC); however, they will not aid in the diagnosis or monitoring of the clinical course of sepsis itself. Currently, from the FDA approved markers for the assessment of sepsis there have been MIP designs for PCT [104], CRP [105, 106] and lactate [107]. In terms of electrochemical MIP based sensor platforms, there is an example for CRP [108]. By far the most abundantly studiedare lactate sensors, most likely due to the lower cost of the target and multiple applications for a sensor of this type, such as in the field of athletic performance. The two sensors described in literature that aim predominantly at utilisation this both use electropolymerisation of 3-aminophenylboronic acid (3-APBA) as their functional monomer for MIP formation, which highlights the importance of selecting suitable monomers for the application [109, 110]. Poly(3-APBA) can be formed on the surface of the transducer *via* oxidative electropolymerisation [111] with the boronic acid functional groups attached to the polymer backbone being free to interact with hydroxyl containing compounds [112, 113].

Zhang et al. [110] focussed on producing a wearable sweat sensor for monitoring muscular performance during exercise, however as the relevant concentration levels are similar it has potential for use in other areas such as septic shock diagnosis [114]. They utilised SPEs as the best flexible working electrode material modified with Ag Nanowires (Ag-NWs, 120 nm diameter and 15 μm length, Fig. 5A) due to their excellent electrical conductivity and adequate flexibility; the MIPs primarily form on the AgNWs due to this enhanced conductivity. The system uses DPV of the ferri/ferrocyanide redox couple for its method of detection which produces a wide logarithmic linear working of 10^{-6} – 0.1 M with a LOD of 0.22 μM, Fig. 5B. This platform shows very promising results, however, there are drawbacks with the sensor being mass producible. Screen printing lends itself excellently to mass production, but electropolymerisation of every electrode for 30 cycles at 50 mV s^{-1} would provide manufacturing challenges; although once production has been completed the device can be marketed as ready to use. Other than this, the results published highlight why electrochemical MIP sensor platforms are a significant area of interest for POCT, as the sensor showed no degradation in performance even after dry storage over 7 months. This explicitly shows one of the main advantages that MIPs bring to POCT when compared with their biological recognition element counterparts such as antibodies.

There have been very few examples of MIP based electrochemical sensors for the detection of inflammatory markers. One report for the detection of interleukin-8 based on MIPs formed on Fe_3O_4 nanoparticles produced a low LOD but very narrow linear working range [115]. A more promising novel approach for detecting interleukin-1β was reported by Cardoso et al. [116] The group has pioneered the use of Eriochrome Black T (EBT) as a functional monomer for electropolymerisation and the formation of MIPs [117]. It possesses several different functional groups that allows for the formation of many non-covalent interactions in addition to its highly extended π system. In the sensor platform for IL-1β, the SPE is coated with PEDOT/4-aminothiophenol to form a linking group between the MIP and the transducer. When formed, the MIP has a significantly negatively charged surface, which in turn leads to a higher charge transfer resistance (R_{CT}) when measured using ferri/ferrocyanide redox couple due to electrostatic repulsions [118]. As binding of the target occurs, the protein's heterogeneous charge distribution lowers the electrostatic repulsion resulting in a measurable decrease in the R_{CT} [119]. This allowed a LOD of 1.5 pM to be obtained with a wide linear range of 60 pM to 600 nM showing its suitability for the application. However, the triple electropolymerised functionalization of the electrode indicates there would be issues in mass production of the device. Additionally, no data has been reported for the lifetime of this sensor and it negatively charged MIPs may not be as durable as uncharged MIPs.

3.3. Cancer

There are a vast array of different types of cancers, the most common of which include lung, breast, colorectal, prostate, skin and stomach [17]. The TNM staging system is a standard method of diagnosing and classifying cancer stages, where T denotes the size of a tumour, N denotes the lymph node spread and M denotes the presence of absence of metastases. This method remains useful for bulk classification but the presence and measurement of individual biomarkers can help with subdividing classes to aid with monitoring, treatment approaches and recovery [120]. There is a vast array of different biomarkers that can be associated with different cancer types; For example, Carcinoembryonic Antigen (CEA) and Epidermal Growth Factor Receptor (EGFR) are both useful markers in colon cancer, where CEA can aid in monitoring,

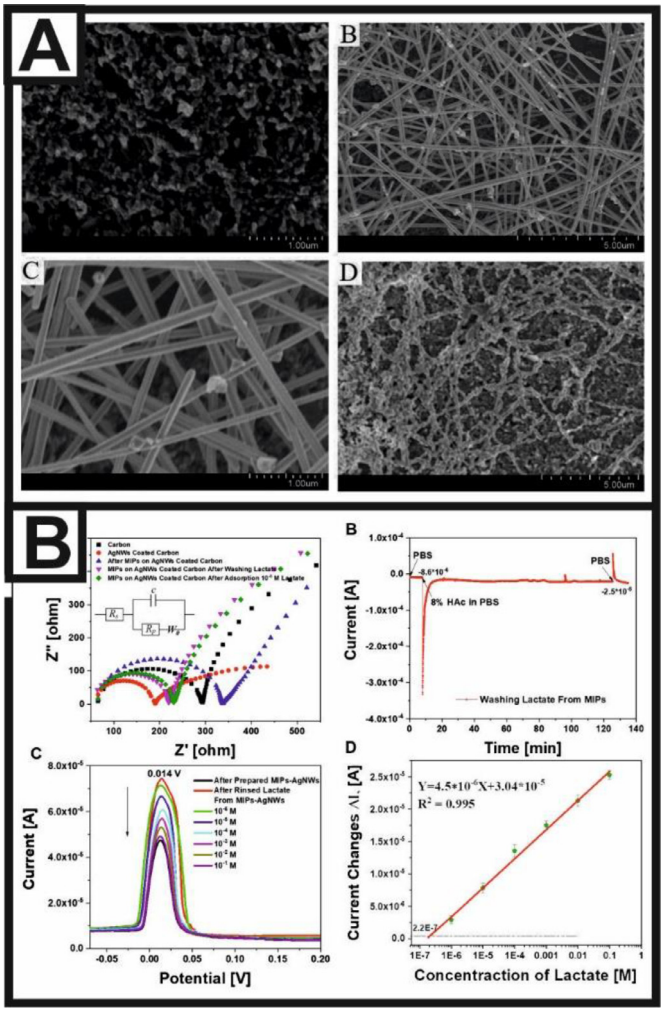

Fig. 5. A) SEM images of the screen-printed carbon working electrode, AgNWs coated on the electrode and MIPs formed on the AgNWs. Reproduced/adapted with permission from ref [110]. Copyright 2020 Elsevier. B) EIS data for the functionalization of MIP electrodes and DPV data for incubation with lactate. Reproduced/adapted with permission from ref [110]. Copyright 2020 Elsevier.

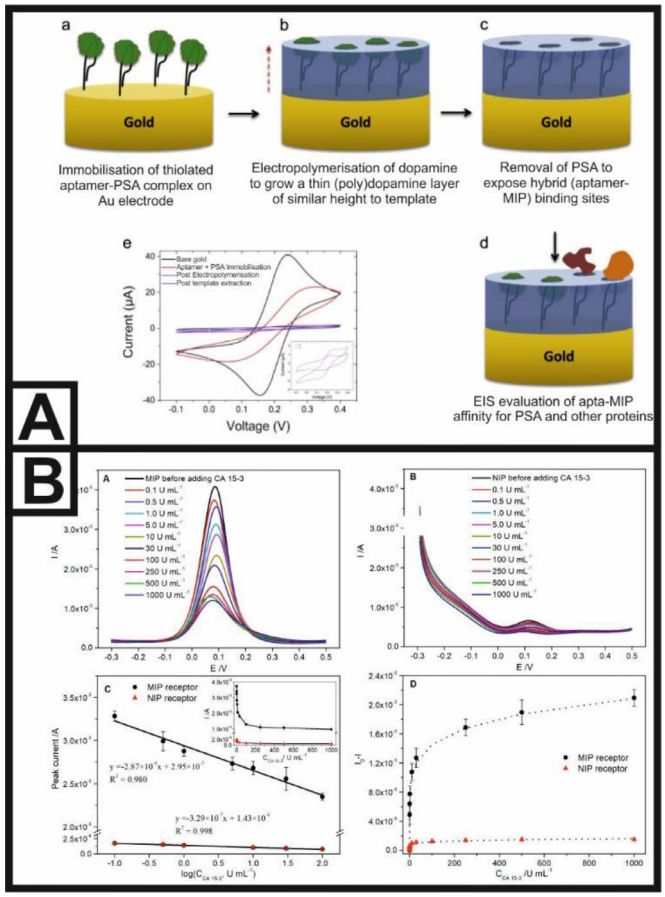

Fig. 6. A) Schematic representation of the sensor fabrication for the aptamer/MIP based platform. Reproduced/adapted with permission from ref [124]. Copyright 2016 Elsevier. B) DPV data in the presence of 5 mM $[Fe(CN)_6]^{3-/4-}$ with the MIP (A) and NIP (B) along with the associated calibration plots (C) and binding isotherms (D). Reproduced/adapted with permission from ref [122]. Copyright 2018 Elsevier.

while EGFR is clinically used to help select appropriate therapies [120]. A summary of some MIP based electrochemical biosensors can be found in Table 2.

Breast cancer is one of the most commonly diagnosed cancers worldwide, with Human Epidermal Growth Factor Receptor 2 (HER2), Cancer Antigen 15–3 (CA 15–3) and CEA being the most widely reported biomarkers [121]. This is also the cancer that has received the most reports of MIP based electrochemical sensors. Two sensor platforms have been reported recently for CA 15–3 by Ribeiro et al. [122] and Pacheco et al. [123], which utilise similar methodologies for the analysis. Both systems utilise DPV as their electroanalytical technique and produce LOD's and linear working ranges at similar levels. The study by Ribeiro does report a wider working range at 0.1–100 U mL^{-1} and slightly lower LOD at 0.1 U mL^{-1}, Fig. 5B. As the systems presented both utilise Au SPEs, DPV and the ferri/ferrocyanide redox couple, the difference between the two is expected to be predominantly due to the choice of functional monomer for the MIP.

Pacheco utilised 2-aminophenol as the functional monomer, which produces a non-conductive polymer network. Using a non-conductive polymer alongside electroanalytical detection methods will always limit the working linear range of a system. In comparison, Ribeiro and coworkers utilised Toludine Blue as their functional monomer which produces a conductive polymer. To achieve this, they created a self-assembled monolayer (SAM) with a toluidine blue tail group to act as a linker between the polymer and electrode surface. This helps to produce the greater working linear range but also adds significantly longer times to platform production. In this way, the system reported by Pacheco could be fully prepared and ready to use in the same time it took for just the SAM to form for the alternative system. Pacheco et al. [125] also report a similar system for the detection of another breast cancer marker (HER2) utilising phenol as their functional monomer. This highlights key parameters that need to be considered when developing a sensor platform suitable for commercialisation as a POCT; the balance between sensitivity, working range and production scalability is vital.

In terms of biomarkers for other cancers, Jolly et al. [124] reported a hybrid MIP/aptamer, Fig. 6A, sensor (the benefits of these systems are discussed earlier) for the detection of prostate specific antigen (PSA). Firstly, the aptamer is immobilized onto the gold surface, then the target is introduced and bound to the aptamer, followed by MIP formation around this complex. The imprinting step here is achieved through electropolymerization of dopamine, which binds readily to electrode surfaces [126]. This sensor platform produced a linear response from 100 pg mL^{-1} to 100 ng mL^{-1} which highlights the strength of these hybrid devices.

As we begin to see more of these hybrid designs in sensor platforms there is a key compromise that must be addressed by researchers; namely, the balance between achieving the relevant biological LODs

and working ranges and, the methodology of sensor preparation and its viability for scalable mass production and cost effectiveness.

5. Conclusions and future outlook

The development of electrochemical biosensors that incorporate MIPs as their recognition element are gaining popularity in research due to the significant benefits that MIPs bring. The ability to tailor a production method to a specific target, the excellent chemical and thermal stabilities, the reduction in the use of animals and the low-cost of polymers mean that they are well suited to the application. There are several examples of MIP based sensors in recent years that match the required LOD and working linear range to be biologically relevant, however the majority of these use complicated production methodologies. In our opinion the greatest challenge in the development of MIP based sensors that are used in real world applications, be that clinical POCT or as home monitoring/diagnosis kits, is the optimization of production methods. This means developing methods that can produce sensor platforms that reach the biologically relevant detection levels, whilst also being a scalable mass production methodology. Overcoming the drawbacks of electropolymerisation (mass production difficulties), bulk polymerization (heterogeneous binding sites) and many other synthetic methods (poor synergy with electrochemical detection) are all key challenges to address. Given the significant advantages that MIPs offer to sensor platforms, we expect this area of research to be of expanding interest in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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